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EXAMINER
HENDRICKS, K

ART UNIT	PAPER NUMBER
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COOLEY, GODWARD, CASTRO,
HUDDLESON & TATUM
5 PALO ALTO SQUARE
3000 EL CAMINO REAL, 4TH FL
PALO ALTO, CA 94306

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

☒ This application has been examined ☐ Responsive to communication filed on _____ ☐ This action is made final.

A shortened statutory period for response to this ection is set to expire 3 month(s), _____ days from the date of this letter.
Failure to respond within the period for response will cause the application to becoma abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☒ Notice of References Cited by Examiner, PTO-892.
2. ☒ Notice re Patent Drawing, PTO-948.
3. ☐ Notice of Art Cited by Applicant, PTO-1449.
4. ☐ Notice of Informal Petent Application, Form PTO-152
5. ☐ Information on How to Effect Drawing Changes, PTO-1474.
6. ☐ _____.

Part II SUMMARY OF ACTION

1. ☒ Claims 1-21 are pending in the epplication.

   Of the above, claims _____ ere withdrawn from consideration.

2. ☐ Claims _____ heve been cancelled.
3. ☐ Claims _____ are ellowed.
4. ☒ Claims 1-21 are rejected.
5. ☐ Claims _____ are objected to.
6. ☐ Claims _____ are subject to restriction or alection requirement.
7. ☒ This application has been filed with informel drawings under 37 C.F.R. 1.85 which are ecceptable for examination purposes.
8. ☐ Formal drawings are required in response to this Office ection.
9. ☐ The corrected or substitute drawings have been received on _____. Under 37 C.F.R. 1.84 these drawings are ☐ ecceptable; ☐ not ecceptable (see explanation or Notice re Petent Drawing, PTO-948).
10. ☐ The proposed eddltional or substitute sheet(s) of drawings, filed on _____. has (hava) been ☐ approved by the axaminer; ☐ disapproved by the examiner (see explanetion).
11. ☐ The proposed drawing correction, filed _____, has been ☐ approved; ☐ disapproved (see explanetion).
12. ☐ Acknowledgement is made of tha claim for priority undsr U.S.C. 119. The certified copy has ☐ been received ☐ not been received ☐ been filed in parent application, serial no. _____; filed on _____.
13. ☐ Since this application apppears to be in condition for allowance except for formal matters, prosecution as to the marits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
14. ☐ Other

EXAMINER'S ACTION

The Group and/or Art Unit location of your application in the PTO has changed. To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 184.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. § 103, the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 C.F.R. § 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of potential 35 U.S.C. § 102(f) or (g) prior art under 35 U.S.C. § 103.

Claims 2-11 and 22 are rejected under 35 U.S.C. § 112, first paragraph, as the disclosure is enabling only for claims limited to methods of producing a Bacillus novo species PB92 of reduced indigenous extracellular protease level, transformed with a mutated B. novo PB92 alkaline protease. See M.P.E.P. §§ 706.03(n) and 706.03(z).

Claim ~~1~~ 22 ~~and thus its dependent claims, are of undue breadth~~ because it is not enabled the broad language ~~for~~ contained therein, due to the recitation of "~~...a protease differing in at least one~~ a protease other than a protease native to said host amino ~~acid from a wild type protease...~~". One of ordinary skill in the art would not be able to determine which of the numerous proteases known (and yet unknown) to select in order to produce a functional system according to the invention. ~~Further, it would~~ require an undue amount of experimentation to ~~determine which amino acid to change within the entire molecule, in order to~~ produce a molecule to ~~function according to the~~ applicant's

~~invention~~.

Claims ~~1~~ 22 and 14 are also ~~of undue breadth~~ not enabled for the recitation of the phrases "Bacillus strain incapable of producing a wild type protease", and "an indigenous protease gene", respectively. The applicant's have not shown that this is possible, and actually part of the invention, for each and every protease gene in each and every Bacillus species, as all are not known nor cloned. Further, the applicant has not shown this to be true for all protease molecules of the particular Bacillus novo species PB92 strain exemplified; only the protease in which the corresponding wild-type gene has been deleted from the Bacillus host are sufficiently expressed upon transformation with the mutated gene, without production of that particular "wild-type" protease.

Similarly, claim 9 is ~~of undue breadth~~ not enabled for the recitation of a "sequence at least substantially similar to that of a Bacillus novo species PB92 protease or a fragment thereof". From the claim, as written, one of ordinary skill in the art would not be able to determine which protease of that particular PB92 species to utilize, which of the numerous known proteases would be sufficient to be "at least substantially similar" to one of the PB92 proteases, or even what fragment of those proteases would be necessary to fulfill the invention. It would thus require an undue amount of experimentation to determine these broad aspects of the claims, and thus they are not properly enabled.

Claims ~~X~~ 2, 9, 12, 14, 17-21, ~~and thus their dependent claims~~, are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 is confusing and/or incorrect for the recitation of "In a method for...", and the use of the ~~term~~ "Bacillus" should be italicized or underlined, consistent with the other claims. The second paragraph of claim 1, in its entirety, is confusing, from the use of "a first DNA sequence" and "a second DNA sequence", etc. It is unclear as to which has been deleted and which inserted; in what chronological order, regarding the "host prior to transformation of a parent of said expression host...".

Claim 2 is incorrect, as the words "is an alkalophilic" are italicized.

Claim 9 is vague and indefinite in the recitation of the phrase "at least substantially similar to that of a Bacillus...protease or a fragment thereof." It is not clear as to how much sequence homology and functional similarity, etc. would be required to be "at least substantially similar" to any of the proteases of that species, or fragments of those proteases.

Claim 12 is vague and indefinite for the recitation of the phrase "coding region and optionally portions of the 5' and the 3'... leaving a sequence...". It is unclear from the claims as

to how much "portions" constitute of the gene, if indeed they are to be deleted, and how much of "a sequence" is left.

Claim 14 is vague and indefinite for the recitation of the phrase "capable of producing", as it is not clear as to the definition of this, and how this phrase defines said species different from any other form of protein-producing strain.

Claim 17 is confusing as to which alkaline protease, the claimed intended protease or "a wild-type", is the one that "differs in at least one amino acid from the wild-type protease produced". Claim 18 improperly recites "said Bacillus strain" from claim 17, but lacks a clear antecedent basis for this in the independent claim (17). Claims 19-21 are confusing and/or incorrect in the recitation of "proteases according to claim 16", as claim 16 is directed to a Bacillus strain, and does not discuss any protease, let alone more than one. Claims 20-21 are of improper format, for the recitation of "Use of one...". It is suggested that these claims be written using acceptable claim language, thus setting forth positive method steps.

The following is a quotation of the appropriate paragraphs of 35 U.S.C. § 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless --
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1, 3, 6-7, and 10-11 are rejected under 35 U.S.C. § 102(b) as being clearly anticipated by Bott et al.

Bott et al. teach of mutant Bacillus strains incapable of producing subtilisin (alkaline protease) or neutral protease. Then cloned carbonyl hydrolases (proteases) are used to transform the host for sole expression of the mutated proteases. Multiple copies of the hydrolase gene may be incorporated into the host genome. "This is facilitated by bacterial strains which are particularly susceptible to homologous recombination. [pg.13]" Thus, the interfering genes may be deleted from the Bacillus strain containing a homologous gene. "All that is needed is for regions of the residual DNA from the deletion mutant to recombine with homologous regions of the candidate host."

These mutant strains "are advantageous in the fermentative production of products ordinarily expressed by a host that are desirably uncontaminated with the protein encoded by the deletion gene. [pg.15]" This group may consist of "mutant host genes exhibiting substantial pretransformation homology with those of the host. These include mutations of procaryotic carbonyl hydrolases such as subtilisin and neutral protease..." "The mutation per se need not be predetermined [pg. 17]", including site-directed mutagenesis to facilitate higher expression or activity. Finally, Bott et al. discloses these isolated alkaline proteases within a detergent composition for use in the processes of laundry. Thus, the claims, as written, are clearly anticipated by the prior art, and are not deemed novel.

The following is a quotation of 35 U.S.C. § 103 which forms the basis for all obviousness rejections set forth in this Office action:

> A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.
>
> Subject matter developed by another person, which qualifies as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

Claims 2, 4, 5, 8-9, and 14-21 are rejected under 35 U.S.C. § 103 as being unpatentable over Bott et al. in view of Van Eekelen et al. Bott et al. is herein incorporated as cited in the above rejection.

Van Eekelen et al. discloses the transformation of Bacillus host strains with vectors comprising two or more copies of a gene encoding a polypeptide of interest. Specifically, one of the two genes described is for the high alkaline protease of an (asporogenic) alkalophilic Bacillus novo species PB92. The use of homologous recombination or illegitimate recombination for the eventual gene amplification is described. "For example in the case of Bacilli as host organisms, the genes encoding extracellular enzymes or genes involved in sporulation can be used as target sequences. Integration of DNA sequences in these

genes will generally inactivate the gene. Loss of expression of the gene can then be monitored and used for the selection of the desired recombinant strain. [pg. 9]" It is desirable to use a host cell with "reduced degradation of the desired product. [pg.13]" "The host cell can also be a mutant of an organism which produces the polypeptide of interest which itself, however, is a non-producer. Where the polypeptide of interest is a protease or an amylase, preferred strains include Bacillus novo species PB92...", etc.

Thus, it would have been obvious to one having ordinary skill in the art to combine the information provided by the two references in order to produce the claimed invention. Bott provides the general method for which to produce Bacillus strains incapable of producing certain desired proteases, wherein the transformation of these same hosts with a mutated "wild-type" protease gene results in the production of that single protease, essentially free of interfering proteases. Van Eekelen discloses the gene for Bacillus novo species PB92 high alkaline protease, and the use of this alkalophilic Bacillus host, with reduced degradation of the product, for the expression of these genes. Thus, it would have been obvious to use the detailed system of Bott et al. in a similar Bacillus species for the expression of a given, indigenous alkaline protease. The mutation of this protease, "differing in at least one amino acid from the wild-type protease", would also be within the ordinary skill in the

art, performed by similar methods disclosed by Bott et al.

Further, claims 17-21 are viewed as obvious over the prior art cited. The production of these particular mutated high alkaline proteases, substantially free from other proteases, is an inherent production resultant of the system of Bott et al. Also, these enzymes may be easily purified by methods common in the art, to insure their isolation from other alkaline proteases. Again, the use of the enzymes within detergent compositions in a laundry process has been shown by Bott et al., and would be an obvious step to include. Thus, these claims are seen as obvious over the prior art cited, and are not deemed patentable.

Claims 12 and 14 are rejected under 35 U.S.C. § 102(b) as anticipated by or, in the alternative, under 35 U.S.C. § 103 as obvious over Fahnestock et al.

Fahnestock et al. disclose that "Bacillus strains having reduced levels of extracellular protease are produced by replacing the native chromosomal DNA sequence comprising the gene for an extracellular protease, such as subtilisin, with a partially homologous DNA sequence having an inactivating DNA segment inserted therein." The alkaline protease gene is inactivated by inserting a DNA fragment of chloramphenicol acetyltransferase (CAT) into the protease gene. Using homologous recombination, the original, functional gene is deleted. Also, the replication function of the cloning vector is inactivated,

yielding a reduced-protease strain. "While subtilisin is the most abundantly produced Bacillus exoprotease, it will be readily appreciated that the procedures described herein can also be employed to inactivate other exoprotease genes in the Bacillus chromosomal DNA, thereby producing strains having even further reduced extracellular protease levels. [column 4]" These strains were also produced to be neutral protease negative. The resultant "strains carrying the inactivated apr gene are excellent candidates for use as hosts for the expression and secretion of heterologous genes. Finally, the limitation of "an alkalophilic Bacillus strain" does not render the claim patentably distinct from the anticipated similar method of Fahnestock, per se. The systems are the same, and both used with Bacillus organisms. Thus, the claims are not deemed patentable in view of the prior art.

Claims 13 and 15-16 are rejected under 35 U.S.C. § 103 as being unpatentable over Fahnestock et al. in view of Van Eekelen et al. These two references are herein incorporated as cited in a previous rejection above.

It would have been obvious to one having ordinary skill in the art to combine the information provided by Van Eekelen, concerning the specific alkalophilic Bacillus novo species PB92 and its protease(s), for use within the identical system disclosed by Fahnestock, for producing a Bacillus with reduced

extracellular protease levels. Thus the claims are deemed obvious over the prior art.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Keith Hendricks whose telephone number is (703) 308-4314.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Elizabeth C. Weimar

ELIZABETH C. WEIMAR
SUPERVISORY PATENT EXAMINER
ART UNIT 184

kdh
September 9, 1991